CELL-COUNTING TECHNIC: A STUDY OF PRIORITY.

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To the man investigating any particular phase of cell-counting, this paper presents a classified and annotated bibliography which aims to be both up to date and complete, unlike all other articles I have seen, which mainly represent those features favored by each writer. To the practitioner are presented reminders of the methods available, with summaries indicating which seems to be preferable for simplicity and accuracy.

The earliest cell count seems to have been done October 6, 1851, and published in 1852 by K. Vierordt. He made nine erythrocyte counts on himself with the remarkably accurate average of 5,174,400 cells. His method of "microvolumetry" was to make a very fine capillary tube with an internal diameter of about 0.1 mm., but accurately known, and a length of at least 5 mm., though these were not graduated. With this capillary he took a drop of blood, measured its length by comparison with a glass micrometer (divided into $\frac{1}{3}$ mm.) on the microscope stage and thus was able to calculate the size of the drop. He then blew it out into a streak of gummy diluting and preserving fluid on a slide, mixed and smeared it and finally counted the entire spread with the aid of a finely squared micrometer eye-piece. The result was sufficiently accurate but the procedure too tedious, requiring three hours or more.

Since this invention of indubitable importance and originality a multitude of modifications have been put forward whose claims as to both value and priority have been considerably debated.

The best valuation of them and in general of the technic of blood-corpuscle enumeration is the monograph by Bürker, "Zählung und Differenzierung der körperlichen Elemente des Blutes," in Tigerstedt's *Handbuch der physiologischen Methodik*, 1912. The reasons are: (1) Bürker's ingenuity in having "brought the technic of blood-cell counting to a hitherto unattained accuracy," in the words of vôn Grützner 1912, (2) Bürker's exceptionally extensive experience in actual counting, (3) Bürker's judicial freedom from the critical and anticritical vituperation which obscures some of the issues argued in this field, such as Hohenpolycythemie and the relative value of Potain's pipette in the Thoma-Zeiss modification and in Miescher's modification. The most peppery example is Schauman and Rosenqvist's remark (May, 1900) that Meissen first misquoted them (October, 1899), then in reply to their remonstrance (January, 1900) misquoted them again but differently (February, 1900), and thirdly, begged the question by laying down the law, as he said, "once and for all."

Another sound and careful critical review, which discusses

Bürker's technic and is therefore especially valuable as a complement, is that by Roerdansz in 1913 and 1914. For example, he pointed out that Bürker's complete method uses so much breakable glassware that it involves much loss of time and money, without compensatory gain in value; and this view was sustained by Krotkow in 1913.

Each of these experts, however, like others in this and other fields, shows a bias toward certain methods, not always his own to be sure, but accompanied by a corresponding neglect of other devices or even a summary repudiation of them. Since, however, the experts do not agree on some of the most essential points, even on what to discuss and what to discard, there seems to be room for an article, even "in our days, in which works on the blood shoot up almost like mushrooms from the earth," in the words of Weidenreich in 1909.

This article will accordingly attempt to supplement the existing judicial and somewhat exclusive personal opinions by an inclusive historical review of the men who have contributed to the science of cell-counting. Some students may like to have available a grouped bibliography of the different parts of the method of blood-counting as it is currently used in this country today, and from this to draw their own conclusions. It is true that the technic of this subject is so well established that few are interested in reviewing it, especially as "the counting of the blood corpuscles in counting nets does not exactly belong to the special pleasures of human existence," as remarked by von Grützner in 1912 and cited approvingly by Roerdansz in 1913. In this connection Bürker's experience is interesting: In 1905 he sent questionnaires to all domestic and foreign physiological institutes or departments in seats of learning listed in *Minerva Jahrbuch der gelehrten Welt*, amounting to 202. He received back only 25 (13 German and 12 foreign), *i. e.*, only 12 per cent. Only 11 indicated any special experience with technic.

Furthermore, few men seem to realize the full variety of ingenious devices that have been worked out to relieve this or that small but annoying difficulty. Just now anybody wishing to look up the literature on any particular feature, such as counting-rulings or blood-stickers, in order to select the best for his particular purpose, will, I think, find such a compilation nowhere. This paper then is partly a critical review and partly a historical summary, with the main interest an attempt to discern priority for the various procedures.

Chronological Bibliography. Far the most thorough bibliography extant accompanies Bürker's monograph, already referred to, including 320 titles. It is arranged chronologically, not alphabetically nor in sequence of citation in the text. This order seems worthy of more frequent use, especially to aid in placing workers historically in the development of their subjects and to enable a writer to cite others simply by name and year when dealing with a subject on which a standard chronological reference list has once been compiled.

The bibliography with this paper, therefore, aims not to recite the three hundred and eighty-six references which I have been able to examine but to dovetail onto Bürker's reference list by:

1. Including certain references overlooked by him.
2. Including certain references incorrectly cited by him.
3. Including certain references subsequent to this work of his (1912).
4. Including certain references to cell counts of other body fluids than blood (namely pleural, peritoneal and spinal punctates), of moulds and of bacteria. These amount to more than two hundred additional titles.

Nomenclature. *Names for this Special Field.* In the development of cell-counting technic a great variety of names are found: microvolumetry (Vierordt in 1852), micrométrie (Malassez in 1872), micrometric numeration (Hart in 1881), Hämocytometrie (Pappenheim in 1912), down to blood-corpuscle enumeration or blood-counting (present-day journals like the *Index Medicus* and the *Journal of the American Medical Association*). The term cell diagnosis of Widal in 1900 is more inclusive, but the broadest and best seems to me cell-counting (cytometry).

Terms for the Counting Chamber. Of the instruments used in cell-counting the chamber has received far the most careful attention and modification. The study of its metamorphoses up to the beautifully made and eminently satisfactory appliance of the present day may appropriately begin with a review of the various names applied to it. First we have the Zahlenmikrometer (Welcker in 1853), then the rectangular capillary or capillary cell (Cramer in 1855), globulimetro (Mantegazza in 1865), compte globules à capillaire artificiel or simply capillaire artificiel, counting capillary or capillary tube (Malassez in 1872), hématimètre or chambre humide (Hayem and Nachet in 1875), Hämatimeter (Hayem-Sahli in 1914).

The term most familiar today, h(a)emacytometer (Gowers in 1877, Durham in 1897, Student in 1907, circulars of Levy in 1916, Leitz in 1919, Bausch & Lomb in 1920) is found in the literature about as frequently as the spelling hæmocytometer (Oliver in 1896, Samut in 1909, Buchanan in 1909, Gulland in 1914, Wells and Sutton in 1915, patent of Levy in 1916, Kennedy in 1916).

Another group of similar names includes the Zähl-Apparat or Zähl-Vorrichtung (Abbe-Thoma in 1879), corpuscle-counter (Hart in 1881), Zählkammer or counting chamber (Thoma in 1882), cytometer or cell-counter (Bizzozero in 1887), Blutkörperchenzähler (Oppenheimer in 1889), blood-counter (Kakowski in 1910), Zählplatte or counting table (Grawitz in 1911), Blutkörperchenzählapparat (von Grützner in 1912), Blutkörper-Zählkammer (Roerdansz in 1913).

Then there was the compte-globules à chambre humide graduée (Malassez in 1880), translated as micrometric graduated corpuscle-counter with wet chamber (Hart in 1881). The term cellule à rigole or moat chamber (Gilbert and Lyon in 1884) was applied to Hayem's hématimètre, and the same instrument has also been called a cellule calibrée (Luzet in 1893).

Another group of titles follows: Schlitzkammer, a circular moat slit chamber (Meissen in 1898), double slit chamber (Starcke in 1898), four-slit chamber (Meissen in 1899), parallel-trench slit-chamber (Bürker in 1905) and H-trench chamber (Bürker in 1907).

In sum, the term most generally used today seems to be hemacytometer.

Type of Slide. *Flat Side.* In the beginning was the flat slide of Vierordt in 1852 and Welcker in 1853 and 1854. It was also used later by Mantegazza in 1865, Loewenberg in 1908, Ellermann and Erlandsen in 1909, Geissler in 1911, Thomson in 1912; and for spinal fluid by Widal and Ravaut in 1900, Laignel-Lavastine in 1901 (?), Joffroy and Mercier in 1902 (?), Nissl in 1904, Niedner and Mamlock in 1904, Fischer in 1906, Kafka in 1910, 1911 and 1915, Geissler in 1911 and Szecsi in 1911 and 1912.

Hollow Slide. The kind of slide or counting chamber in common use in the United States and Europe is of the type generally and deservedly credited to Thoma-Zeiss. It may be remarked, however, that while the instrument made by Zeiss of Jena was preëminent before the World War, now the products of the American manufacturers may be considered equally good, if not in fact the best. It is also of some interest that although Abbe in 1879 announced Thoma's recommendations, Thoma's first publication in 1881 appeared under the joint names of (1) an American, Lyon of Norwich, Conn., and (2) Thoma of Heidelberg. The fame of this so-called Thoma-Zeiss instrument was due mainly to its perfection, for its principal features were not new. These features were three: the slide with the chamber proper, the quadrilled ruling and the site of this ruling. Each of these three will now be detailed:

The earliest chamber may be discerned in the capillary cell or rectangular capillary of Cramer in 1855. This consisted of two parallel glass bands cemented onto a slide close to the long edges, and with another thin slide cemented on top of them. This could apparently be filled by capillarity, though the inventor used a glass tube and an ingenious wooden adapter to permit suction. The capillary of Malassez in 1872–73–74 was very similar. This linear chamber was seen later in Alferow's and in Bürker's devices.

The development of the chamber with a circular pit was begun by the invention of Hayem and Nachet in 1875, who took a plate of glass with an aperture of 1 cm. diameter, ground it with emery to be exactly 0.2 mm. thick and cemented it tightly to the slide. This style of chamber has been in world-wide use by Gowers in 1877, Thoma in 1879, Henry and Nancrede in 1879, Malassez in 1880, Hart in 1881 (Gower's modification), Laache in 1883, Karcher in 1897, Einhorn and Laporte in 1902, Helber in 1904, Samut in 1909, Sahli in 1909 and Jörgensen in 1913; and all those who have introduced ingenious amplifications of the original Thoma ruling or substituted for it new nets (see under Style of Ruling), besides the mass of the profession who have not published their observations.

Lyon and Thoma in 1881 added a plate in the center of the pit, leaving a circular moat 3 mm. wide.

The principle of two parallel troughs, instead of the circular moat, to limit the counting area, was introduced by Alferow in 1884. By so doing he gained, like Cramer in 1855, the great advantage of having the cover-glass in good position before inserting the drop of blood and also of being able to insert the drop by capillary attraction. These features were made use of by Bürker in 1905 in his parallel trench chamber and in 1907 in his even cleverer double chamber with two counting islands, forming what might be called an H-trench. Parallel lateral trenches were also used by Liebreich in 1916. The H-trench is not only literally the latest word today, but is generally regarded by all who have used it as the last word.

The circular moat chamber gives too high results compared with the parallel trough chamber, by 5 per cent according to Loewenberg in 1908, 10 per cent according to Bloch in 1912 and Adachi in 1912 and by 7 per cent according to Bürker in 1913. Similar conclusions were reported by Marloff in 1919 and Fritsch in 1920.

Another variety of so-called slit chamber which has never apparently won recognition outside a small group of observers was that projected by Meissen in 1898: a Thoma-Zeiss with a narrow and shallow radial furrow 0.5 mm. deep, to make the chamber independent of barometric pressure. A double slit was offered by Starcke in 1898 and a four-slit cavity by Meissen in 1899. Bürker in 1905 noted that whatever advantage under conditions of changing barometric pressure might accrue to a slit chamber, Cramer's, Alferow's and his own afforded this advantage even more completely than those just mentioned. Fuchs in 1908 and von Koranyi in 1908, who used Bürker's chamber, both speak of it as a slit chamber, but since then the term and the dispute are defunct.

Depth of the Chamber. *Depth of Chamber Proper.*—The earliest chamber, Cramer in 1855, was 0.066 mm. deep. Only one shallower has been proposed, that by Liebreich in 1916, which was 0.025 mm. After Cramer, Hayem in 1875 made his newly invented pit measure 0.2 mm., in which he was followed by Gowers in 1877, Malassez in 1880, Alferow in 1884, Helber in 1904, Fuchs and Rosenthal in 1904, Laruelle in 1906 and Glaubermann in 1913. Two greater depths have been used: 0.222 by Friedlaender in 1897 and Cuzzolini-Durati in 1906 and 0.5 by Nageotte in 1907 for spinal fluids.

Thoma in 1879 reduced the depth to 0.1 mm. in order to permit use of a higher power objective with greater magnification, shorter focus and brighter illumination. This depth has been accepted by all since him with the exceptions here noted.

Bürker in 1905 neatly straddled the breach between the two most popular depths by making the depth of the chamber itself 0.1 mm. and then providing twice that depth for use when cells are scanty (eosinophils, erythroblasts or for spinal fluid) by having made what is described below as a double depth cover-glass.

Thickness of Cover-glass. The cover-slips made for counting chambers have varied from 0.15 mm. (van Walsem in 1915), 0.18 (Lyon and Thoma in 1881, Schröder in 1899, Turban in 1900, Gottstein in 1900), 0.35 (Lyon and Thoma in 1881, Schröder in 1899), 0.40 (Bürker in 1912), which is perhaps the commonest, to the extraordinary limit of 3.3 mm. (Turban in 1900). The most ingenious kind is what may be called a two-depth cover-glass, *i. e.*, one of ordinary thickness, to which is cemented a thinner circlet or flat paster of glass pierced in the center with a hole, being in depth 0.18 mm. (Lyon and Thoma in 1881), 0.15 mm. (van Walsem in 1915) or best just 0.1 mm. (Bürker in 1913). The last named makes the chamber 0.1 mm. deep when placed on the slide with the paster side up, 0.2 mm. deep when with the paster faced down.

Occasion may be taken in passing to mention the recommendation by Bürker in 1911 that the two longer edges of the cover-slip instead of being at right angles to the major surfaces and mat in finish should be rounded and smooth-polished, in order to facilitate more rapid introduction of the blood by capillary attraction. This refinement seems plausible, but has not been taken up so far as I am aware.

Site of Ruling. The counting net may be in one of four situations, as summarized by Alferow in 1884: the ocular, the floor of the chamber, the substage whence it is projected on the floor of the chamber and the ground glass of an affixed microphotographic chamber. The last suggestion, by Alferow himself, and the projection principle of Nachet in 1875 have received no attention. The rivalry has run between the first two methods.

The ocular method was the first in use. The eye-piece was either itself cross-ruled or contained a quadrilled glass plate or a diaphragm, and in the beginning no attempt was made to standardize the tube length or graduate the ocular ruling. It was used for orientation only. This was the case with Vierordt in 1852, Mantegazza in 1865, Loewenberg in 1908, Ellermann and Erlandsen in 1909, von Grützner in 1912 and Thomson in 1912.

Then the eye-piece was not only quadrilled but calibrated, an "ocular micrometer" or "micrometric quadrilled ocular." First by Cramer in 1855, who set his tube so that the field coincided with a hole in a tin band lying on the slide like the guide on a slide-rule.

The area exposed by the hole was 0.172 square mm. and the ocular was ruled into 48 oblongs. Malassez in 1872 and 1873 set his tube with the guidance of an objective micrometer and then labelled his tube length. Standardized oculars were also used by Alferow in 1884, Ehrlich in 1898, Hayem-Sahli in 1909, Bürker in 1911, Metz in 1914 and 1915. Kennedy in 1916 patented a hemacytometer comprising a scale arranged in the eye-piece, having a central inner circular area, surrounded by a central larger area and an annular outer area encircling the central areas and divided radially to form bacteria counting chambers.

The floor was ruled but not graduated in the instrument of Welcker in 1854. The base of the chamber was both ruled and calibrated by Gowers in 1877. This was a priceless step in advance and has been followed by Thoma in 1879, Malassez in 1880, Hart in 1881, Alferow in 1884, Bürker in 1905 and all the proponents of new rulings detailed below. Before proceeding to them it may be of interest to quote from Abbe in 1879, in view of the scarcity and inaccessibility of his exhaustive article on the principles involved in cell-counting: "The application of an objective ruling to the bottom of the counting chamber has the great advantage over the ocular micrometer, of dispensing with the determination of the absolute value of the ocular ruling, which naturally varies for every objective and every tube-length." As would be expected from this theory in practice the ruling is in most cases preferred on the slide.

Agasse-Lafont in 1920 suggested rulings both for the island and for the floor of the moat.

Style of Ruling. The kinds of counting nets that seem of possible interest today are those which follow Thoma, in matter of time and more or less in style. The majority were originally ruled in the circular chamber, but are now cut in the parallel trench chamber or might be if so desired.

The classical ruling of Thoma in 1879 and 1881, aptly called the Thoma cross by Breuer 1902, was a gridiron of 1 mm. side, divided into 400 squares which were set off by double lines into sixteen groups of twenty-five squares each. Later the double lines were shifted to make twenty-five groups of sixteen squares each; this accounts for the puzzling difference between some Thoma rulings.

Zappert in 1892 and 1893 drew a line across each of the four ends of the cross which protrude 1 mm. beyond the central square millimeter, enclosing with these four lines 9 sq. mm. instead of 1. For counting white cells in the blood, and particularly in less cellular fluids, this increase of the ruled area was immediately recognized as invaluable and has since been adhered to by the majority of ruling inventors.

Elzholz in 1894 modified Zappert's net by adding three pairs of vertical lines through the left column of 3 sq. mm. and also through the right column. This he illustrated, but incorrectly according to

Türk in 1902, who then gave a figure which he asserted to be correct; not, however, convincingly. Thus the subject is confused by contradictions.

Friedlaender in 1897 depicted an entirely different type without the extra line at the border of every fifth square, which was such a feature of Thoma's grating. This new net for leukocytes measured 4.8 mm. on the side instead of 3 like Zappert's or Elzholz's, and was divided into 16 parts, making 256 squares, with a total counting volume of 5.11 c. mm.

Durham in 1897 modified Zappert's field by dividing each of the four corner square millimeters into four squares.

Türk in 1902 apparently built on the basis of Elzholz's by adding to the verticle double lines horizontal pairs, thus showing in each corner square millimeter sixteen squares, separated by rectangles and tiny squares between the double lines. This net largely displaced Thoma's, and now is being in turn displaced by Neubauer's, which shows also 16 subdivisions of each corner millimeter but more simply separated by single lines.

Breuer in 1902 introduced, and Sahli in 1911 and Morawitz in 1912 commended, a development of Zappert's by the division of each of the eight peripheral square millimeters into 36 rectangles.

Brünings in 1903 used a net something like that of Malassez in 1872. It was cut in a facet in the bore in the center of an elaborate U-shaped pipet, with an ampulla at each end, *i. e.*, a combined chamber and mixing pipet.

Fuchs and Rosenthal in 1904 enlarged the field from 9 to 16 sq. mm., which they lined in new fashion, subdividing each square millimeter into 8 or 16 squares or rectangles. No illustration is seen in the original paper, but may be found in the section on cerebrospinal fluid in Frazier's *Surgery of the Spine*, 1918.

Bürker in 1905 divided each of the 9 sq. mm. into 16 squares by paired lines, just as was done by Türk for the four corner millimeters.

Simon in 1906 followed Bürker's in appearance, but the squares were somewhat larger, and the complete net rather smaller, 4 sq. mm.

Nageotte and Levy-Valensi in 1907 planned for spinal fluid a huge field, 15 mm. on the side, ruled off into 225 sq. mm.

O. Neubauer, date not discovered but first cited by Meyer and Rieder 1907, aimed at simplicity like Durham, 1897, but instead of making only four parts of each corner millimeter made sixteen. This is the same number as proposed by Türk, 1902, but has the advantage of the greater clarity inherent in using single rather than paired dividing lines. The result is the only net which has rivalled the popularity of Türk's and which is now rapidly outstripping it in this country.

Sahli in 1909 and 1914, perhaps earlier, first experimented with concentric rings around a square, with for calibration an objective micrometer scale of 1 mm. divided into 100 parts.

Kakowski in 1910 for urinary cell counts gave an account of a large round island with a surface area equal to 4 sq. mm., with for calibration a line 3.1 mm. long, finely subdivided.

Dunger in 1910 and 1911 believed that he was simplifying the counting not only of blood but also of exudates, transudates, and especially spinal fluid, by surrounding the Thoma cross by two differently ruled "mantels." He also increased the sides of the field from 3 x 3 mm. to 7 x 7 and $\frac{1}{7}$ mm., the additional seventh being to yield the round figure 50 for easy calculation.

Predtetschenski was credited with a new net design by Goriaew in 1910.

Goriaew in 1910 and 1914 (elsewhere transliterated from the Russian as Goriaeff and Gorjajew) enlarged Predtetschenski's net from 4 to the usual 9 sq. mm., but though the novel design has been taken up by Pappenheim in 1912 and Greene in 1917, its neglect by the rest of the world makes detailed description superfluous.

Howard in 1911 had made by Bausch & Lomb, as a counting chamber for moulds, a Thoma-Zeiss cell with a center disk of $\frac{3}{4}$ inch (18.75 mm) instead of $\frac{1}{4}$ inch (6.25 mm.), as usually furnished.

Morris "some time between 1909 and 1911" (personal communication) was the first to suggest the double Bürker chamber with double Neubauer ruling, which he obtained from Zeiss through A. H. Thomas. This combination he urged again in 1913 and 1916, and today it seems to be the favorite in this country, and increasingly so. It is a great convenience whenever one has occasion to make a count to find the same ruling in general use.

Klyuchareff in 1912 proposed the combination of Bürker's counting camera with Predtetchenski's net.

Pappenheim in 1912 produced a variation which he called the Gorjajew-Pappenheim ruling, based on an interpretation of the former's work, which was erroneous, according to Goriaew in 1914.

Roerdansz in 1913 alternated larger and smaller squares than those of Thoma. The engraving is said to be cut in a new manner so as to show as bright violet.

Schirokauer in 1914 applied Türk's ruling to Bürker's later chamber, what I have called the H-chamber.

Bass in 1915 illustrated a thoroughly novel and beautifully simple ruling. The total field he, however, reduced to 4 sq. mm. The chamber is made in this country by Bausch & Lomb. If it covered 9 sq. mm., like that of Neubauer, it would, I think, prove a rival.

Liebreich in 1916 suggested, instead of squares, oblongs 10 x 0.1 mm. There were 60 of these millimeter areas. Half were further divided by horizontals into four and all 60 were subdivided by verticals into a hundred parts.

Zappert-Ewing, date unknown but first cited by Wood in 1917, consists of a substitution of three single lines for the three pairs of lines used by Elzholz in 1894 to divide each corner square millimeter into four rectangles. This simplification by using single instead of double lines may be compared to Neubauer's simplification of Türk's ruling.

Agasse-Lafont in 1920 proposed a new ruling for the ditch in the circular chamber, in order to give ample space for spinal fluid counts.

Summing up, the style of ruling most popular today is the Neubauer or, as it is sometimes called, the Zappert-Neubauer.

The Best Counting Chamber. From the preceding sections it will be plain that the best cell-counting chamber is generally considered to be the "double Bürker" (slide) "double Neubauer" (ruling).

This combination may be had in this country from the following dealers: Bausch & Lomb, of Rochester, N. Y. (their own make), E. Leitz, of New York City (own make), A. H. Thomas, of Philadelphia (Max Levy's make), and Spencer, of Buffalo (also, as I am given to understand, Levy's make). These names are given in alphabetical order, not in any order of merit. Each manufacturer exhibits modifications, some of distinct value. The underlying principles, and the claims as to results attained, are worth attention, but will not be discussed at present. In the different makes each worker, according to his taste, can secure the standard polished finish (yielding Newton's rings) or the mat (dull) grinding; raised or flush supporting strips; supporting strips of one piece with the slide or cemented onto it; the ruled strip cemented onto the slide or cemented and also recessed into the slide, and additional parallel troughs for convenience of contact.

Any of these makes has seemed satisfactory for practical work. For publishable observations, however, one cannot overlook the reports by Henry and Nancrede in 1879, Hart in 1881, Alferow in 1884, Lyon in 1917, A. H. Thomas in 1920 (personal communication) and the U. S. Treasury, Bureau of Standards (personal communication), as to occasional errors in instruments of the best manufacturers as purchased in the open market. For example, the last-named authority may be quoted: "The need of demanding certified apparatus is understood when we say that the percentage of hemacytometers conforming to the specifications varies from 99 per cent of those sent in from one manufacturer to 68 per cent of those sent in from another manufacturer, the error practically always being in the depth of the chamber." Accordingly a student of finer points in cell-counting would do well to send his chamber, cover-glasses and pipets to the Bureau of Standards for test as to accuracy of compliance with the tolerances adopted by that Bureau, "upon request, in 1918, after considering the accuracy obtainable by the manufacturers by the use of proper methods and reasonable care."

Newton's Rings. Perhaps the most interesting question in connection with this choice of modifications in the slide is whether to use the mat (dull ground glass) finish or whether to adhere to the classical smooth polish which yields Newton's rings. They were first observed by Hooke in 1665 in mica plates, or as it was then called Muscovy glass, and also in soap-bubbles and in other substances. He remarked that "We are able from a colorless body to produce several colored bodies." These he called "fantastical colors." The study was continued by Sir Isaac Newton in 1675 and 1704 with, in the words of Rayleigh in 1911, "his accustomed power, and by him most of the laws regulating these phenomena were discovered. He experimented especially with thin plates of air enclosed by slightly curved glasses, and it should be noted that among slightly curved glasses belong most cover-slips, though supposedly plane, and the colored rings so exhibited are usually called after him 'Newton's rings.'" The name he used was "colors of thin plates." Young in 1802 showed that the formation of these colors was due to the interference of light reflected from the two surfaces of the plates. Hence the name often used, "interference rings."

These rings, often only stripes or bands, have been absolutely required by many of the most careful students of blood-counting technic, notably Abbe in 1879, Thoma in 1881, Schröder in 1889 and Turban in 1900. Brünings in 1903 observed (1) that neglect to produce the stripes was accompanied by an error of 9 to 13 per cent, which was confirmed by Bürker in 1904; and (2) that the depth of the chamber was the same, provided the color bands were present, no matter whether they were broad or narrow, dry or moist; and this too was confirmed by Bürker in 1913. The 386 publications I have read do not all discuss the rings, but among the large number which do, not one fails to insist on their importance. Among the teachers of clinical blood-work whom I have consulted all but one prefer the standard smooth finish. In Guthrie's words: "With the ground-glass type the assumption of good approximation may frequently be correct, but there is no indicator to show that this is so." I agree strongly because I fail to see how the mat finish betrays a tiny foreign body of dust or lint.

On the other hand it must be admitted that the mat finish is heartily favored by one experienced worker whom I consulted as well as used in three of the four American makes of hemacytometer. It is true that in the use of polished chambers care is not always exercised to see that Newton's rings appear, and many workers not familiar with interference phenomena do not understand their significance when they do appear.

Other Counting Methods. The parallel trench chamber of Bürker, without ruling, was used by Bürker in 1907, von Grützner in 1912 and by Metz in 1914.

The hematocrit was used by Hedin in 1891, Daland in 1891, von Jaksch in 1892 and 1893, Lederer in 1895, Schäfer in 1898, Schmincke in 1911, Epstein in 1916, and others whom I have not hunted up, owing to the small part hitherto played by this instrument as a substitute for true cell-counters. It has, however, its uses, and in the judgment of many might well be applied more generally to check red cell counts and to determine the proportion of cells to plasma. A similar volume per cent method was published by Harvey in 1920.

Counting, so called, by an entirely different method is seen in the chromocytometer of Bizzozero in 1887 and the haemocytometer of Oliver in 1896.

Dreyer in 1921 reported a simple procedure for the accurate estimation of blood cells without the use of a counting chamber. He dilutes the blood with a permanent standard suspension of hen's corpuscles, which being elliptical and nucleated are easily counted simultaneously. He claims that this method is cheaper, quicker and less fatiguing than the usual methods.

Total Contents of Chamber. The amount of fluid held in the counting space varies markedly. The smallest volume, as nearly as I can make out, was 0.002 c.mm. (Alferow in 1884). Then 0.008 cmm. (Hayem and Nachet in 1875), 0.011 (Cramer in 1855), 0.02 (Gowers in 1877), 0.04 (Vierordt in 1852, Alferow in 1884) and 0.1 (Thoma in 1879, Meissen in 1898, Starcke in 1898, Turban in 1899, Einhorn in 1902, Samut in 1909, Bloch in 1912 and Roerdansz in 1913). Then 0.2 c.mm. (Brünings in 1903, Helber in 1903), 0.25 (Thomson in 1912), 0.4 (Goriaew in 1910, Pappenheim in 1912, Bass in 1915), 0.6 (Simon in 1906) and 0.9 (Zappert in 1892, Elzholz in 1894, Durham in 1897, Türk in 1902, Breuer in 1902, Bürker in 1905, Neubauer in 1907 and Goriaew in 1914). Then 1.0 c.mm. (Malassez in 1880), 1.5 (Liebreich in 1916), 1.8 (Bürker in 1907, Galambos in 1910, Schirokauer in 1914, Morris in 1916, Levy in 1916), 3.2 (Fuchs and Rosenthal in 1904, Laruelle in 1906), 3.72 (Bürker in 1912), 5.0 (Oliver in 1896, Dunger in 1910), 5.11 (Friedlaender in 1897). Then 10 c.mm. (Loewenberg in 1908, Ellermann and Erlandsen in 1909, Kakowski in 1910, Glaubermann in 1913), 20 (Geissler in 1911, von Grützner in 1912), 40 (Kakowski in 1910, Geissler in 1911). Nageotte's cell was probably the biggest, the contents being variously stated as 10 cmm. (Nageotte and Riche in 1907), 50 or 100 (Bürker in *Tigerstedt* in 1912) or 225 c.mm. (Nageotte and Lévy-Valensi in 1907).

Placing the Cover Glass. The importance of placing the cover glass rapidly, in order to avoid unequal distribution of the cells, was early appreciated. At first the cover was set in a hinged clamp which could be laid on quickly and smoothly, by Malassez in 1880. The later clamps of Gowers in 1877, Alferow in 1884 and Bürker in 1907 were not hinged and merely prevented movement of the slip once it was in place.

Roerdansz in 1913 made a clamp into which the cover-glass was slid after the blood had been dropped in the chamber. A similar but simpler method, the author of which I have lost, was to slip the cover half-way across the circular chamber to deposit the drop, then slip the cover the rest of the way.

To dodge the great difficulty of making the drop neither too big nor too small, many have used the method of placing a good-sized drop, then slipping the cover-glass right *through* the drop, with the result that the "excess will rise on top of the cover and jump across the moat." This seems to have been first published by Eustis in 1913, whereupon rival claims were immediately put forward. Rea in 1913 stated that the method had been used in the Pepper Laboratory of Hygiene since 1899. Barnett in 1913 ascribed the credit to L. D. Zulick, and Kraemer in 1913 reported that the method had been current in the Jefferson Medical College since 1904.

All these methods, however serviceable, seem likely to be superseded by the spread of the Bürker chamber because it allows placement of the cover-glass with the obtaining of Newton's bands prior to the deposition of blood.

Pipets. *Types.*—Of all the pipets for measuring, or measuring and mixing, blood (or other cellular fluids of the body) and diluting (or preserving or staining) solutions the earliest was Vierordt's in 1852. This was calibrated in diameter but not in length, which had to be laboriously determined beside a micrometer laid on the microscope stage. It held about 0.04 cmm.

Welcker in 1854 introduced a pipet holding a fixed volume, 4 c.c., and readable with the naked eye, as have been all pipettes since.

There are two main classes of pipet: (1) Separate capillaries for measuring the sample of body fluid and for the diluting solution, and these incidentally are further complicated by the addition of a mixing vessel in most methods except those in which the mixing is performed directly on the counting slide; (2) a combined pipet for not only measuring but mixing in an ampulla the cellular fluid and the diluting solution.

Separate Pipets. Of this type were Vierordt's and Welcker's, just mentioned, and later those of Mantegazza in 1865, Hayem and Nachet in 1875 (the only widely used design), Mayet in 1888, Ellermann and Erlandsen in 1909, Sahli in 1909, von Grützner in 1912, Ellermann in 1913, Krotkow in 1913 and Sahli in 1914. Of them all Hayem's alone is conspicuous, as one peruses the literature, for wide recognition, mostly in France. But even it has had nowhere nearly the popularity of the combined pipet about to be described.

Single Mixing Pipet, Mélangeur, Mixer. The prototype of this invaluable device was what Roerdansz in 1914, called "the lovely idea of Malassez and Potain." The latter invented his mélangeur in 1867 but no description was published until the paper of Malassez in 1872. The modern modification is what is known as the erythrocytometer or simply the red pipet.

Sörensen in 1876 pointed out that the upper mark should be close to the ampulla. Thoma in 1879 enlarged the bore slightly to facilitate cleaning; his pipet was about 8 cm. long; the ampulla contained 0.5 to 1.0 c.c., and as a whole his Misch-Vorrichtung was so reliably calibrated by Zeiss that it promptly earned the pre-eminent popularity which after forty years still persists. Hart in 1881, noting that Gower's counting chamber was 10 per cent inaccurate as to depth (which had already been reported by Henry and Nancrede in 1879), recommended recalibrating Potain's mélangeur to compensate. This procedure was obviously rendered unnecessary by the precision of several manufacturers since Zeiss set the pace at Thoma's request.

Thoma made a still further contribution in 1882 by having for white cells a similar Mischgefäss, diluting 1 to 10. This has been frequently called a leukocytometer or simply a white pipet.

Stierlin in 1889 credited to O. Wyss a modification of Potain's mélangeur with two ampullæ, but this has received no further recognition.

Rieder in 1892 gave an account of his shortening, for convenience sake, of the pipet to two-thirds the Thoma-Zeiss, and of his new graduation 1 and 21, *i. e.*, a dilution of 1 to 40.

Miescher in 1893 among other inventions defined two which, though not in general use, have been highly commended by such practised observers as Lederer in 1895, Kündig in 1897, J. Karcher in 1897, S. Karcher in 1897, Veillon in 1897, Suter in 1897, Schauman and Rosenqvist in 1898, Müller in 1910, Bürker in 1911, Roerdansz in 1912, Krotkow in 1913, Emerson in 1913 and Wood in 1917. These improvements seem unfortunately unknown in this country except to those few who have had an opportunity to use the Fleischl-Miescher hemoglobin pipets, which show the same features. These are:

1. Ring graduations instead of the usual short horizontal lines. Krotkow in 1913 thought these ring marks even more legible when cut as incomplete circles, in the gap of which the meniscus should be read.
2. Accessory graduations, "Hilfstriche," just 1 per cent above and below each mark.

Brünings in 1903 proposed an entirely strange pipet with two ampullæ and between them a counting field engraved on a flat facet in the lumen.

Roerdansz in 1912 elaborated the Thoma-Zeiss pipet with a second ampulla, using the first for diluting only and the second for mixing. This device he further developed in 1914. It goes sometimes by the name of the Fleischauer-Zeiss pipet, after the two firms who manufactured it for him.

Liot and Poussin in 1918 and Harvey in 1920 used ordinary Pasteur capillary pipets.

In conclusion of this section it may be said that by far the most popular pipet today is the single mélangeur of Potain-Malassez in the more accurately made model of Thoma-Zeiss. Further, that the Miescher modifications of the latter deserve wider use.

Automatic Pipets. The next attempt at improvement, both in point of novelty and of time, was this attempt to dispense with judgment by the eye as to when the meniscus touched the mark, and therefore with the invariable anxiety and the frequent error associated. The three-way cock in the gas analysis pipet of Geppert in 1885 had already been adopted for an automatic urine pipet by M. Cremer (nature unknown despite search of the *Index Medicus* back to the first volume in 1879) and was modified by Voit in 1895 for blood-work. May in 1903, in turn, altered Voit's instrument by the addition of a second three-way cock. Hirschfeld in 1909 added a mixing ampulla and had a pipet each for red and white cells. Roerdansz in 1914 reported an "automatic mixing pipet" which followed closely that of May.

The overflow principle as a rival of the three-way cock was first set forward by Hirschfeld in 1911, who took the idea from the Ueberlaufpipette "Mikra" devised by Weichardt in 1910 for his epiphanin reaction for syphilis. Hirschfeld's device seems to have got no notice except condemnation from Roerdansz in 1913. Thomas in 1910 made a simpler automatic pipet by applying to a delivery milk pipet a T-tube, an appliance which might perhaps be as advantageously used with a blood pipet. Wright in 1914 illustrated an overflow pipet, also Harvey in 1920.

Summarizing this section it may be remarked that these refinements have won no place in practice, however valuable they may be to occasional research workers.

Aspirators for Pipets. To govern suction more accurately than is easy for many with the usual mouth method, many contrivances have been described.

The throttle is the simplest. A. E. Wright in 1897, 1912 and 1914 effected resistance to too rapid suction by drawing out a capillary tube to a fine point. An analogous construction was inserted by Boggs in 1907 and by Bürker in 1912 into the blood pipet suction tube. Hirschfeld in 1909 indicated the even easier way of inserting a bit of cotton.

A rubber bulb or teat or camera bulb was introduced for bacteriological pipets by Robert Koch in 1890, who also was the first to use a hole (in the rubber) in order to control the vacuum. The same idea was recommended by Benmosche in 1906 (without the hole) and with the control hole by Beck in 1896, Boggs in 1907, Münzer and Bloch in 1909, Portmann in 1909, Klemensiewicz in 1910, Permin in 1911 and A. E. Wright in 1912 and 1914.

Suction by means of a screw clamp passed around a rubber teat or around a bit of tubing plugged at one end was applied by Gabritschewsky in 1891 to a pipet much like Potain's mélangeur; and also by Gaertner in 1892, Grijns in 1894, Eijkman in 1894 and 1898 and by Levy in 1897.

The same principle was applied somewhat differently when Wieck in 1909 made a frame with a metal wheel and roller which squeezed the rubber tube, filling or emptying the pipet according to the direction in which the roller was moved. This Präzisionssauger was noticed by Grawitz in 1911 and Bloch in 1912. A similar device was independently put forward by Maddox in 1913.

Syringes were initiated by Ebstein in 1903, who fastened the syringe to the blood pipet by means of a set-screw. Galli in 1904 also employed a syringe, with the plunger further elaborated by a screw which permitted slighter movements as well, what we might call a fine adjustment. Küster in 1906 arranged for delicate suction by putting on the plunger a knob which moved in a spiral groove on the metal stub of the barrel and attached his aspirator by a rubber washer and perforated closing screw which turned down on the washer to make it fit pipets of different sizes. Hirt in 1908, independently of Galli, used two plungers, one inside the other, each with a micrometer screw, one for coarse and one for fine adjustment. Weichardt in 1908 used a micrometer screw like Galli and also made another syringe which he attached to the pipet by a short piece of thick rubber tubing. Küster in 1909 modified his previous syringe so that it could be either pulled for coarse adjustment or turned in a slot for fine adjustment. Lautenschläger was credited by Koch in 1910 with having made some kind of sucker; probably this was the Mikropipette regulated with a micrometer screw manufactured by F. & M. Lautenschläger, of Berlin, for Weichardt in 1910. Stroschein was also credited by Koch in 1910 with having melted a Luer glass syringe on to a Thoma pipet. Koch's own device in 1910 looked like a movable lead-pencil holder slipped over the upper end of the pipet and held there by an internal rubber grip, and was in fact a metal syringe, the suction piston of which was moved by a button sliding along the side of the barrel. Engelmann was credited by Bloch in 1912 with a microsyringe, but how this too differed from earlier devices is also not clear from his article nor have I been able to find the original report despite a search of the volumes of the *Index Medicus* for the years 1913 back through 1908. Van Walsem in 1914 put foward a syringe working with a terminal thumb-screw, in principle like the fine adjustment part of the design by Galli in 1904. Saxon and Drummond in 1915 illustrated an apparently practical "aspirator:" a metal syringe with incidentally a flat flange projecting at right angles from each end in order to cap the pipet for carrying.

Cheaper than a syringe was the durable and so-called automatic suction pipet or syringe credited to Voigt and illustrated by Keim in 1906. This seems to be a development of Voigt's (1900) Lymphebläser for smallpox vaccine work, but embodies all the features independently urged by the writers below for blood-work, namely, an external test-tube sliding up and down outside the top end of the pipet for suction, made snug by a surrounding bit of rubber tubing 1 cm. wide and 2 mm. thick, and internal prolongation of the pipet which allowed use of the overflow principle. For serological use the idea was expatiated on by Wolff in 1908. The device was applied to blood pipets by Pappenheim in 1909, who recommended it also for the automatic pipet of Hirschfeld in 1908. Independently shortly afterward Portmann in 1909 used the same kind of sliding barrel made snug by a ring of rubber tubing over the upper end of the Thoma pipet, which he had prolonged to receive it; and he also applied to this a lateral control hole similar to that used by Koch in 1890 in his rubber bulb and first applied to a glass suction instrument in the blood viscosimeter of Hess in 1907. Stroschein was credited by Fuhrmann in 1910 with practically the same scheme. The next step of progress, in accuracy though not in simplicity or cheapness, was made by Pappenheim in 1911 and 1912, who ground the glass like the barrel and plunger of a "Lührschen" (Luer?) syringe. In 1912 he further recommended, in order to make the vacuum incomplete so as not to aspirate too rapidly and forcibly, that the grinding be not perfectly snug; and later still, in 1912, he substituted for this loose grinding the control hole as used by Portmann.

Other automatic pipets or Sicherheitspipetten, the original descriptions of which I have not been able to see, are those of Kapeller in 1907, Meyer (quoted by Keim in 1906), of Gaffky and of Wassermann (quoted by Kempff in 1910); of Dafert, of Kühn and of Wollny (illustrated in catalogue of Vereinigte Fabriken für Laboratoriumsbedarf, Berlin, n. d.).

In summary of this section it may be observed that none of these ingenious inventions has in practice displaced the classical bit of rubber tubing 0.5 cm. in diameter by about 15 to 30 cm. long.

Pipet Carriers. Devices for carrying pipets fall into two groups, cases and caps. Cases have been described (*a*) for pipets and chamber, by the various manufacturers and (*b*) (which is ordinarily more useful) for pipets and diluting fluids by Malassez in 1872, Sutton in 1911 and Haden in 1920.

Caps began apparently in the shape of ordinary elastic bands, as noted by Seymour in 1910; or the mouth end of the suction tubing drawn over the point, as mentioned by Hartz in 1915; or a rubber band cut from a 3½ inch automobile inner tube, as specified by Putnam in 1919. In the case of each of these the frequency of puncture by the sharp end of the pipet led to the introduction of hard objects, such as caps. Seymour in 1910 took gray rubber tubing, such as the thinner-walled variety often used for suction, and cut it to a length slightly less than that of the pipet; occluded the ends by hard rubber plugs and sliced out half the tube lengthwise to within about 1 cm. of each end; then by stretching what remained he slipped it easily over the pipet. This has served me, and many another, well; and seems *a priori* as effective, durable, simple and cheap as any subsequent scheme.

Bunnell later in 1910 used two BB cartridge shells fastened together with a piece of wire and a rubber band. Roerdansz in 1912 had caps of glass ground to fit and relied on the snugness of grinding to keep them in place. Van Walsem in 1914 similarly pushed a small cork stopper onto each end of the pipet, following the familiar trick of guarding the ends of needles or hatpins. Croy in 1914 followed Seymour and Bunnell in employing a rubber connection between the caps, which in his case were two disks of any thin metal. Saxon and Drummond in 1915 also used metal caps but joined them by a spring, superimposed on their aspirator for filling the pipet. McJunkin in 1919 recommended, much like Van Walsem, small soft-rubber stoppers pushed on to each end of the pipet.

Actual stoppers, rather than caps, were introduced by Katzenstein in 1911 by simply inserting the small rubber plugs from serum vials.

Summarizing, the best capping device seems to me Seymour's plugged tubing.

Pipet Shakers. Little in 1917 illustrated a complex electrical motor driven machine for shaking two blood-mixing pipets. Putnam in 1919 much more simply attached the pipet to an ordinary electric buzzer bell and Haden in 1920 showed another electrical machine simpler than Little's and holding four times as many pipets.

Methods of Cleaning Pipets. The technic of removing clotted blood and even of the deposit that occurs periodically with the best of care has been discussed by Türk in 1902, Benmosche in 1906, Bürker in 1911, Thomson in 1912 and Morris in 1913.

Quick Method of Drying Pipets. When a mechanical air draft is not available, a rubber bulb has been reported as useful by Gray in 1921.

Miscellaneous Modifications of Pipets. Galli in 1904 enclosed his instrument in a glass mantle to prevent dilation from the warmth of the hand. The same idea, with the addition of circulating water, recurs in the "Mantelspritze" of Determann in 1905 and of Münzer and Bloch in 1909.

Bürker in 1907 emphasized that the tip of the pipet when angular, as is usual, should be rounded off with emery in order to yield a neater drop.

Thomson in 1911 and 1912 offered a suggestion which seems sensible but has not yet been taken up, namely, that blood pipets be made, like clinical thermometers, with a white opal background of milk glass, and with the front edge bevelled as a magnifier.

Schroeder in 1914 showed a figure of a tiny metal mirror made to slide on a ring along the pipet in order to facilitate reading the calibrations.

Blood Needles. The history of blood-stickers, needles, lances or lancets is confusing owing to (1) the scanty descriptions and indistinct illustrations given in the literature, and to (2) the loose use of some terms. Blood lancet, for example, seems to have indicated sometimes an instrument with a true lanceolate or spear point, sometimes a narrow surgical knife or lancet and sometimes categorically any sticker. Blood needle also may mean an ordinary sewing taper-point needle, a surgical two- or three-edge cutting needle, a glover's three-edge cutting needle (usually shorter cutting edges than a surgical needle), a Hagedorn or Boldt flat needle with a single bevelled cutting edge.

The earliest pricker I have found may serve as an instance of this vagueness, for the nature of its point is not clear from either the text or the cut in the paper by Gowers in 1877.

A sheath or guard with a screw to fix it at varying distances from the tip, and so regulate the amount of blade exposed, was introduced by Malassez in 1880. This fundamental idea is seen in most of the instruments since proposed, namely, in those of Alferow in 1884, Reichert in 1889, Francke in 1889, Daland in 1891, Bensaude in 1893, Oliver in 1896, Ries in 1904, Bürker in 1912, Zapf in 1913, Van Walsem in 1914, Sahli in 1914 and Boldt, date unknown.

A spring lancet was arranged by a spiral wire inserted within the handle, in order to make the stab sudden yet smooth, by Alferow in 1884, then used by Reichert about 1889 and improved by Francke in 1889. The latter, "Francke's nadel," has generally received the credit from those who have written on stickers, and it has been subjected to a number of changes. Ries in 1904 made the point removable and had a space for spares within the distal end of the handle. Boldt and Moore, like Ries, had both the regulable sheath and removable point, but introduced a different spring release. Müller in 1910 recommended that the "Lanzette" of the Francke "Schnäpper" should be semicircular instead of pointed in order to render bleeding freer; and for the same purpose Bürker in 1912 altered the tip from lance shape to that of a broad chisel. Zapf in 1913 made the tip of Francke's needle of platinum-iridium so that it could be disinfected in the actual flame. Van Walsem in 1914 changed the tip to a rather large sewing needle, English No. 4; he is the only author I have discovered who is satisfied with the small amount of blood secured with a taper point. Greene in 1917 substituted for the lance tip a trocar point; the three edges of this, being short and stubby, have seemed to me much less satisfactory than those of a glover's or a surgical needle.

A signet ring, concealing a pricker which looks in the illustration like a true spear point, was projected by Schottelius in 1907.

For the tip of the instrument, leaving out of consideration the handle, more than a dozen kinds of point have been recommended, as follows:

Point with One Edge. 1. Hagedorn in 1881, 1882 and 1885, straight surgical needle. This has a single bevelled cutting edge, which is more easily kept sharp than most other types of edge. The shaft is flat, unlike all other needles suggested; and is long (varying from 4.5 cm. up to 9.5 cm.); both these features make the needle stiffer, stronger, easier to hold and to sharpen. It has been commended by Sutton in 1911, Hastings in 1912, Morris in 1913, Webster in 1914 and McJunkin in 1919.

2. Clean steel pen with one nib broken off. Wright in 1897, von Domarus in 1912, Morris in 1913, Webster in 1914, Williams in 1915.

3. Semicircular end. Müller in 1910.

4. Chisel end. Bürker in 1912.

5. Piece of broken cover-glass. Greene in 1917.

6. Small "lance," one-edge like a narrow scalpel or knife-blade. Gowers in 1877 (?), Malassez in 1880, Bensaude in 1893, Champy in 1913, Webster in 1914, Stein in 1917.

Point with Two Edges. 7. True lance or spear point with two edges. Gowers in 1877 (?), Alferow in 1884, Reichert in 1889, Francke in 1889, Daland in 1891, Oliver in 1896, Ries in 1904, Schottelius in 1907, Zapf in 1913, Sahli in 1914, Swan, date unknown, Webster in 1914 and 1920.

8. Regular surgical straight needle. This title is illustrated in Kny-Scheerer's catalogue as two-edged but *non*-cutting. Favored by Bass in 1910.

Point with Three Edges. 9. Trocar point. This has the shortest cutting edges of any triangular needle, as they extend only about a quarter of the total length of the needle. Katzenstein in 1911, Greene in 1917.

10. A glover's needle or a surgical straight shaft triangular cutting needle or a Keith's abdominal No. 6, are all practically the same. The cutting edge extends about a third of the total length. Probably the most widely used blood needle at present.

11. Bonney's straight needle has three still longer cutting edges, extending about half the total length. It has not hitherto been recommended for use as a blood sticker.

Hollow Tip. 12. Paracentesis needle. Wieck in 1909.

Taper Point. 13. Ordinary cambric or larger sewing needle. Van Walsem in 1914.

Summing up: As the object in view is free flow of blood without the necessity of squeezing the ear the best sticker is that handiest for each worker. I like a large Hagedorn needle.

Methods of Recording. *Record Blanks.* Schemata or blank forms for noting the count of individual fields, with spaces for totalling and for computing percentage differential counts, were outlined by Bürker in 1907 and 1911; his form has been commended by several, especially favorably by Müller in 1910.

Osmond in 1910 had a slate, or a ground-glass plate ruled and labelled permanently in ink, and noted the counts in pencil.

Watters in 1913 pictured graphic charts for leukocytes, showing total counts, differentials and daily variations.

Schilling in 1913 designed an elaborate blank with the headings not only printed but concisely defined by cuts of the corpuscles meant to be included under each title.

Sahli in 1914 printed tables for calculating percentages.

Simon in 1918 illustrated some charts.

Recording Machines. A mechanical instrument for "noting and automatic calculation of blood and other cells" was claimed by Tojbin in 1909 and 1912 to be "the first counting and calculating machine." This "Cytax" bristled with knobs and cost 140 marks. It seems to have been noticed only by Schall in 1912, who proceeded to devise an instrument which he thought simpler, and by von Putkowski in 1913, who used the original.

Schilling in 1911 and 1912 illustrated and wrote at length on his differential leukocytometer. This consisted of 100 steel balls and several metal slots, with graduations at the back of the slots for direct reading off of the number of balls in each slot. This, though rather bulky, would seem, especially if equipped with 200 shot, worthy of trial as a time-saver by those who have occasion to count cells frequently. In 1913 Schilling described his "neue Differential-Zähltafel."

Van Walsem in 1914 recommended (1) an ordinary typewriter, using each of the lower row of keys for a particular variety of white cell, recording blindly without taking the eye off the microscope and afterward counting the different letters; and (2) his Zähllineal, which was a ruler with slits to guide one's pencil, so that here too the eye need not leave the microscope.

Cummer and Dexter in 1915 illustrated a small wooden box to hold upright four "counting machines" which look like the watch-like tally machine in use at admission gates.

Number of Digits to Use. In recording counts more figures are generally used than necessary, and that without signifying any greater accuracy, as pointed out by Bürker in 1911 but since neglected. Three numerals are ample, the rest should be ciphers.

Leukocyte Count. *Total White Count.*—This procedure seems to have been initiated by Malassez in 1874 and since him studied by the following: Gowers in 1877, Thoma in 1882 (modified for leukocytes the dilution of the red pipet, giving the instrument so universally used ever since), Alferow in 1884 (distinguished white cells by raising the tube to lengthen focal distance), Toison in 1885 (first to make fluid permitting counting of white cells in the same pipet with reds; an ideal pursued by Schüffner in 1911 and Türk in 1912), Reinert in 1891 (counted visual fields with a known setting of tube length), Zappert in 1892 (first satisfactory ruling), Rieder in 1892, Elzholz in 1894, Friedlaender in 1897, Durham in 1897, Einhorn and Laporte in 1902 (white count directly from smear, by noting the ratio of whites to reds and counting only the reds in chamber), Türk in 1902, Breuer in 1902, Joffroy and Mercier in 1902, Engel in 1902 (differentiation of whites by refraction of light, much like Alferow), Bürker in 1905 and in 1907, Ellermann and Erlandsen in 1909, Dunger in 1910, Galambos in 1910, Hill in 1912 and Roerdansz in 1913.

Differential White Count. This seems to have been first been done in the smear by Einhorn in 1884. followed by Krönig in 1899 (on spinal fluid), Widal and Ravaut in 1900 (also on spinal fluid), Simpson in 1906, Sheaff in 1912, Schilling in 1913, Nieuwenhuijse in 1914 and Johns in 1914. These writers, it may be mentioned, discussed technic rather than interpretation.

The use of a stain in the mixing pipet with subsequent differential counting of the whites in the chamber was introduced by Zappert in 1892. The idea was worked over by Elzholz in 1894, Zollikofer in 1900 (Kämmerfarbung), Türk in 1902, Riebes in 1905, Dunger in 1910, Schüffner in 1911, Hill in 1912, Zapf in 1913, Lenzmann in 1913 and Gifford in 1920.

White Count Simultaneously with Red Count. The attempt to perform both counts in the red pipet was made by Toison in 1885, Zappert in 1892, Türk in 1912, Zapf in 1913 and Lenzmann in 1913. The latter pointed out that "The attempt to differentiate the white blood cells in the counting chamber, and in this way to make an exact count of the different forms . . . . failed always, owing to the difficulty of staining the white blood cells differentially and simultaneously rendering the red blood cells invisible." Zapf had tried to stain the whites and afterward to destroy the reds. Lenzmann experimented with a reagent to be used with the differential stain to harden the white cells, after which he destroyed the reds with acetic acid. His choice ended with 0.3 per cent corrosive sublimate and 5 per cent formalin in water.

Platelet Count. This subject is of interest mainly to special workers, involves little apparatus notably different from that already covered, and has been recently reviewed both as to methods and results by Zeller in 1919, Degwitz in 1920, Gram in 1920 and Thomsen in 1920.

Cell Distribution on Counting Fields. *Red Cells.* In the examination of this important topic we may refer to Malassez in 1880, Reinert in 1891, Brünings in 1903, Student in 1907 and Bürker in 1904, 1907 and 1913. The latter found by his own counts "Colossal errors . . . through the unequal distribution of cells on the bottom of the counting chamber," and also found these errors easy to avoid by laying on the cover glass first and afterward inserting the drop of blood by capillary attraction. Later the subject was touched on further by Roerdansz and Jörgensen in 1914.

White Cells. The distribution of the leukocytes in the counting chamber is a matter of significance, though less so than with the red cells, owing to the smaller number of them; their distribution in the stained smear is of more import. Zollikofer in 1900 noticed the tendency of certain kinds of white cells to be collected in the thicker parts of the smear, namely, at the two sides and at the end of the slide, and also the further inequality due to the destruction of cells in the thinner part of the spread. Türk in 1902 and Zapf in 1913 described the inequalities somewhat differently. Schilling in 1913 suggested that these two tendencies be counteracted by counting in a "meandering line," zigzagging to and fro from edge to center, in a manner resembling crenelated battlements or a mattress suture. Nieuwenhuijse in 1914 discussed this suggestion.[1] Johns in 1914 also has something to say about the distribution of leukocytes. Obviously consideration must be given to the way in which the smear was made, whether with cover-slips (Ehrlich), slides (Jansco-Rosenberger) or "thick drop" (Ross and Thomson). The cover glass method has been most recently urged by Meulengracht in 1920, as yielding an evener distribution of leucocytes.

Mechanical Stage. Accessory stages to permit moving the counting slide evenly and systematically past the nosepiece have been reported by Welcker in 1853, Hayem in 1889, Canon in 1892, Klein in 1893, Friedlaender in 1897, Bezançon and Labbe in 1904, Detto in 1906, Sahli in 1909 and Geissler in 1911. Today the perfected movable stage may be obtained from the various manufacturers under their names, not those of the inventors.

Counting Diaphragm. Diaphragms to set limits, fixed or adjustable, to the counting field have been recommended by Cramer in 1855 (perforated piece of tinfoil lying on the slide; contrast this with later diaphragms inserted in the eyepiece), Koch in 1878, Alferow in 1884 (metal or paper), Ehrlich in 1898 (Blenden-ocular, a Huyghenian eyepiece with a square stop which is capable of being varied in size by means of an external knob), Einhorn and Laporte in 1902 (metal with a square hole subdivided by two crossed threads), Bürker in 1905, Larrabee in 1906 (simple but practical, cardboard with a hole cut out), Samut in 1909 (the aperture cut to coincide with the "four squares of the central platform of the counting chamber"), von Grützner in 1912 (three square openings lying in one radius of the eyepiece, which is meant to be rotated to cover the field), Sheaff in 1912 (piece of end of a slide cut off, a small square ruled in its center to afford a sufficiently restricted field for counting leukemic bloods, and this glass diaphragm placed in ocular), Metz in 1914 (a small square in center, subdivided by crossed lines, much like the device of Einhorn and Laporte), Schirokauer in 1914 (used the so-called Thoma-Metz counting ocular just described), Van Walsem in 1915 (tin square cut to correspond to the medium-size square of the Thoma ruling, *i. e.*, one of the sixteen squares separated by the double lines).

Dark Field. The proper illumination of the counting chamber is important, owing to the fact that the cells are in most cases unstained. The dark field has been discussed by Raehlmann in 1905, Neumann in 1907 and 1908, Posner in 1908, Dietrich in 1908, Wiener in 1908, Leva in 1909, Bürker in 1913. The last named has emphasized the value of Abbe's substage condenser and diaphragm in accentuating the colorless ruling of the chamber.

Binocular Microscope. This instrument is beginning to be seen in medical laboratories. Its most striking advantage seems to be the greater comfort of using both eyes. Its history and characteristics have been extensively discussed by von Rohr in 1920.

Theoretical Mathematics. The higher mathematical side of cell counting has been taken up by Abbe in 1879, Lyon and Thoma in 1881, Lyon in 1881, Gram in 1884, Reinert in 1891, Miescher in 1893, Liebreich in 1905, Zuntz in 1905, Student in 1907, Bürker in 1912, Ellermann in 1913, Aebly in 1919, Alder in 1919, Rohrer in 1919 and Kilgore in 1920. Under this heading are considered both the empirical error in the handling and observation on the part of the worker and also the so-called probable error in any given series of observations.

The empirical error should be no larger than 4 per cent; that is, according to Emerson's simple rule, a student should begin by counting blood from the same normal person daily at the same hour until successive results differ by less than 200,000. Experienced men report better figures: Cramer in 1855 claimed 1.6 per cent, Abbe in 1879 felt that 3 per cent was assured if one counted 5000 cells, Reinert in 1891 got 4.6 per cent, Türk in 1902 got 3 per cent, Bürker in 1912 got 1.8 per cent and Ellermann in 1913 got 3 per cent. A different "error" is attained in part, owing to usage of the word by different men for a different mathematical constant. For example, two counts satisfying Emerson's rule would be 5,000,000 and 5,200,000. The second varies from the first by 2 in 50, giving a 4 per cent error; or under another interpretation one might say each count varies from their mean, which is 5,100,000, by 1 in 50 or 2 per cent. Any of these results is obviously excellent compared with the 5 to 10 per cent error put up with, according to Rohrer in 1919, in most clinical methods.

In practice the most important use of the mathematical calculation of the probable error is to decide whether a variation in two consecutive counts is truly significant or whether it must be discarded as falling within the limits of the known likelihood of variation: three times the "probable error." For the actual method of calculation, reference must be made to one of the articles cited or to a modern text-book such as Yule's *Statistics*.

Diluting Solutions. This subject is so large that the following list, unlike the rest I have compiled, makes no attempt to be complete. Of the many writers included in the bibliography mention may be made of: Hayem in 1875, Gowers in 1877, Toison in 1885, Mayet in 1887 (eosin for staining reds brilliantly; osmic acid), Muir in 1891, Zollikofer in 1900, Simon and Spilmann in 1904 (further study in eosin for red cells), Riebes in 1905, Rous in 1907, Dunger in 1910, Pitfield in 1911, Hill in 1912, Gelbart in 1912, Zapf in 1913, Callison in 1914, Jörgensen in 1914, Van Walsem in 1915, Diner in 1917 and McJunkin and Charlton in 1918 (new stain for differential count).

The consensus of opinion favors Hayem's solution for red cell counting, *e. g.*, Bürker in 1905 and 1911, Sternberg in 1905, Boston in 1905, von Domarus in 1912, Türk in 1912, Champy in 1913, Morris in 1913, Sahli in 1914, Williams in 1915, Webster in 1916, Wood in 1917, McJunkin in 1919 and Naegeli in 1919. In the community where I live Gower's fluid is the one generally used, apparently owing to the influence of Cabot's monograph in 1904. Toison's solution is recommended by Greene in 1917 and Simon in 1918.

For white cells the favorite remains the simplest—acetic acid, 0.5 per cent.

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CHRONOLOGICAL BIBLIOGRAPHY OF CELL-COUNTING TECHNIC.

This list is supplementary to Bürker's, discussed on page 527, and with that covers the literature completely, I believe. For the reader of this paper it would be more convenient to have the two lists consolidated, but space forbids.

1665

Hooke, R.: Micrographia, London, 1665, Observation 9: Of Fantastical Colours, pp. 48–68.

1704

Newton, I.: Opticks, London, 1704, Advertisement 1, p. 3; and Observation 2: Rings of Colours, p. 121.

1802

Young, T.: On the Theory of Lights and Colours, Bakerian Lecture, Philosophical Transactions, London, 1802, p. 12.

1853

Welcker, H.: J. G. Dingler's Polytechnisches Journal, 1853, 130, 267, Table 4, Figs. 21 and 22.

1855

Cramer, A.: Nederlandsch Lancet, 1854–1855, Ser. 3, 4, 453–478, with plates: also inadequately summarized in five lines in Jahresbericht d. ges. Med., 1855, 1, 34, and in Schmidt's Jahrbücher d. in- und ausländ. ges. Med., 1855, 95, 14.

1865

Mantegazza, : Del globulimetro, Milano, 1865; reviewed by Malassez, L.: De la numération des globules rouges du sang, Paris, 1873, p. 10.

1867

Potain, : cf. Malassez 1872 and 1873.

1872

Malassez, L.: Comptes rendus et mémoires de la Soc. de Biol., 19 Oct. 1872, 5e Serie, **4**, (**24**), 213; and 23 Nov. 1872, S. 5, vol. **4**, p. 236.

1876

Keyes, E. L.: Am. Jour. Med. Sc., Jan, 1876, **71**, 17.

1878

Koch, R.: Deutsche med. Wochenschr., 26 Oct. 1878, **4**, 531; also in Gesammelte Werke, Leipzig, 1912, **1**, 80.

1879

Henry, F. P., and Nancrede, C. B.: Boston Med. and Surg. J., April, 10, 1879, **100**, 489.

Thoma, R.: cf. Abbe, E.: Sitzungsberichte der Jenaischen Gesellschaft für Medicin und Naturwissenschaft für das Jahr 1878, Sitzung am 29. November; Jena 1879; also in Jenaische Zeitschrift für Naturwissenschaft, 1879, **12**, (N. F. **5**), Supplement-Heft, xcviii.

1881

Hagedorn, : Verhandl. d. deutsch. Gesellsch. f. Chir., Berlin, 9 Apr. 1881, **10**, pt. 2, 55; also Arch. f. klin. Chir., 1881, **26**, 783.

1882

Hagedorn, : Verhandl. d. deutsch. Gesellsch. f. Chir., 3 June 1882, **11**, pt. 2, 172; also in Arch. f. klin. Chir., 1882, **28**, 522.

1884

Einhorn, M.: Inaugural-Dissertation, Berlin, 21 Mch. 1884.

Gilbert, A., and Lyon, G.: Archives générales de médecine, Nov. 1884, viie S., **14**, 583, and Dec. 1884, viie S., **14**, 713.

1885

Hagedorn, : Medical News, Mch. 14, 1885, **46**, 305.

Geppert, : Gasanalyse, Berlin, 1885, p. 13.

1887

Mayet, : Comptes rendus Acad. des Sciences de Paris, 14 Nov. 1887, **105**, 943; and Lyon médical, 27 Nov. 1887, **56**, 407.

1890

Koch, R.: Deutsch. med. Woch., 13 Nov. 1890, **16**, 1029.

1891

Gabritschewsky, G.: Centralbl. f. Bakt., 5 Sep. 1881, Abt. I., Orig. **10**, 249.

Daland, J.: Fortschritte der Med., 15 Oct. 1891, **9**, 823; and 1 Nov. 1891, **9**, 867.

1892

Gaertner, G.: Allg. Wiener mediz. Zeitg., 8 Nov. 1892, **37**, 513.

Bethe, M.: Morphologischen Arbeiten, ed. by Schwalbe, G., Jena, 1892,**1**, 207.

1893

Luzet, C.: in G. M. Debove and C. Achard's Manuel de médecine, Paris, 1893, **2**, 467.

1894

Gryns, : Zittingsversl. d. Kononkl. Akad. v. Wetensch., Amsterdam, 24 Feb. 1894, cit. by Hamburger, H. J.: Osmotischer Druck, Wiesbaden, 1902, **1**, 443.

Eijkman, C.: Jaarversl. v. h. laborat. v. pathol. Anat. en Bacteriol. te Weltevreden 1894; cit. by Hamburger.

1895

Timofejewsky, D. J.: Centralblatt für allgem. Pathol., 23 Feb. 1895, **6**, 108.

Voit, F.: Zeitschrift für Biologie, 1895, **31**, (N. F. **13**), 179.

1896

Eijkman, C.: Arch. f. path. Anat., 9 Mch. 1896, **143**, 450.

Oliver, G.: Lancet, June 20, 1896, **1**, 1703.

Beck, G.: Aerztliche Technik (Beilage zur Zeitschr. f. Krankenpflege), July 1896, **18**, 100.

Weiss, J.: Hämatologische Untersuchungen, Wien, 1896.

1897

Wright, A. E.: British Med. Jour., May 15, 1897, **1**, 1214.

Durham, H. E.: Edinburgh Med. Jour., 1897, (**44**), N.S. **2**, 351.

Levy, J.: Aerztliche Polytechnik (Beilage zur Zeitschr. f. Krankenpflege), 1897, **19**, 149.

1898

Schäfer, E. A.: Text Book of Physiology, Edinburgh and London, 1898, **1**, 149.

.1899

Krönig, G.: Verhandl. des Congr. f. inn. Med., 11–14 April 1899, **17**, 569.

1900

Voigt, : Aerztliche Technik (Supplement zur Zeitschr. f. Krankenpflege), Jan. 1900, **22**, 12.

Widal, F., and Ravaut, P.: Comptes Rendus et Mem. Soc. de Biol., 30 June 1900, **52**, 648, 651, 653; and 13 Oct. 1900, **52**, 838.

1901

Laignel-Lavastine: Bull. et mem. Soc. med. des Hôpitaux, 21 June 1901, 3^e S., **18**, 704.

1902

Einhorn, M., and Laporte, G. L.: Medical News, Apr. 19, 1902, **80**, 741.

Joffroy, A., and Mercier, E.: Jour. Mental Pathology, Oct.-Nov. 1902, **3**, 58.

1903

Kramer, J. D.: Am. Jour. Insanity, July 1903, **60**, 107.

Brion, A.: Centralblatt f. Allgemeine Pathologie und Pathologische Anatomie, 15 Aug. 1903, **14**, 609.

Ebstein, E.: Münch. med. Woch., 15 Sep. 1903, **50**, 1601.

Ewing, J.: Clinical Pathology of Blood, Phila., ed. 2, 1903, 35.

1904

Nissl, .: Centralbl. f. Nervenheilkunde, April 1904, **27**, (N.F. **15**), 225.

Niedner, O., and Mamlock, G. L.: Zeitschr. f. klin. Med., 1904, **54**, 111.

Fuchs, A., and Rosenthal, R.: Wiener med. Presse, 30. Oct. 1904, **45**, 2061 ff. For illustration of ruling see Frazier, C. H.: Surgery of the Spine, New York and London, 1918, p. 177.

1905

Rachlmann, E.: Wiener med. Woch., Jan. 1, 1905, **55**, 30.

Determann, .: Verhandl. der Kong. f. inn. Med., 14 Apr. 1905, **22**, 477.

Bürker, K.: Münch. med. Woch. 9 May 1905, **52**, 912.

Bunting, C. H.: Johns Hopkins Hosp. Bulletin, June 1905, **16**, 222.

1906

Larrabee, R. C.: Boston Med. and Surg. Jour., May 24, 1906, **154**, 577.

Einhorn, M.: The Post-Graduate, July 1906, **21**, 670, translation of his German Inaugural-Dissertation, Berlin, 21 March 1884.

Simpson, C. E.: Jour. Michigan Med. Soc., July 1906, **5**, 367.

Bunting, C. H.: Jour. Exp. Med., Oct. 12, 1906, **8**, 625.

Murphy, E. V., and Strodl, G. T.: N. Y. Med. Jour., Oct. 27, 1906, **84**, 814.

Detto, C.: Zeitschr. f. wissensch. Mikroskopie, 1906, **23**, 301.

Keim, L.: Lehrbuch der Bakt., Stuttgart, ed. 3, 1906, p. 175.

Laruelle, L.: Jour. de Neurol., 1906, **11**, 576.

Kuster, E.: Centralbl. f. Bakt., 1906, I. Abt., Orig. **40**, 271.

1907

Boggs, T. R.: Jour. Am. Med. Assn., Jan. 5, 1907, **48**, 47.

Student, .: Biometrika, Feb. 1907, **5**, 351; rev. in Folia haematologica, March 1908, **5**, 430.

Kapeller, .: Aerztl. Polytechnik, Mch. 1907, **29**, 47.

Schottelius, M.: Aerzt. Polytech., Apr. 1907, **29**, 56.

Rous, F. P.: AM. JOUR. MED. SC., Apr. 1907, **133**, 567.

Neumann, A.: Zentralbl. f. Physiol., 18 May 1907, **21**, 102.

Hess, W.: Münch. med. Wochenschr., 6 Aug. 1907, **54**, 1590.

Nageotte, J., and Riche, A.: in Cornil and Ranvier's Manuel d'histologie pathologique, Paris, ed. 3, 1907, **3**, 211.

Nageotte, J., and Levy-Valensi, .: Comptes rendus et mem. de la Soc. de Biol., 7 Dec. 1907, **63**, 603.

1908

Hirt, R.: Aerztl. Polytechnik, June 1908, **30**, 81.

Wiener, E.: Wiener klin. Woch., 19 June 1908, **21**, 910.

Neumann, A.: Wiener klin. Woch., 2 July 1908, **21**, 989.

Weichardt, W.: Verhandl. d. Gesellsch. deutsch. Naturforscher, 23 Sep. 1908, **80**, 588.

Fuchs, R. F.: Sitzungs-Berichte der physik.-med. Sozietät in Erlangen 1908, **40**, 206.

Dietrich, .: Folia haematol., 1908, **6**, 190.

Wolff, M.: Centralbl. f. Bakt., 1908, i. Abt., Orig. **46**, 648.

1909

Hirschfeld, H.: Berl. klin. Woch., 8 Mch. 1909, **46**, 499.

Hirschfeld, H.: Die Deutsche Klinik, Berlin and Vienna, 1909, **12**, Ergänzungsband **1**, p. 253.

Münzer, E., and Bloch, F.: Med. Klinik, 14 Mch. 1909, 5, 400.
Pappenheim, A.: Berliner Hämatologische Gesellschaft, 11 May 1909, in Folia Haematologica, June 1909, 8, 96.
Leva, J.: Berl. klin. Woch., 24 May 1909, 46, 961.
Wieck, O. A.: Zeits. f. Krankenpflege; Aerztl. Polytechnik, Oct. 1909, 31, 145.
Samut, R.: Lancet, Nov. 13, 1909, 2, 1424; rev. Jour. Am. Med. Assn., Dec. 11, 1909, 53, 2039.
Buchanan, R. J. M.: The Blood, London, 1909, 65.
Küster, E.: Centralbl. f. Bakt., 1909, i. Abt., Orig. 50, 490.

1910

Osmond, A. E.: Jour. Am. Med. Assn., Jan. 8, 1910, 54, 124.
Seymour, M.: Jour. Am. Med. Assn., Feb. 5, 1910, 54, 467.
Thomas, J. B.: Jour. Industrial and Engineering Chem., July 1910, 2, 330.
Stitt, E. R.: Philippine Jour. of Science, July 1910, 5 B, 233.
Weichardt, W.: Zeitschr. f. Immunitätsforschung, 27 July 1910, Orig. 6, 649.
Bunnell, S.: Jour. Am. Med. Assn., Aug. 13, 1910, 55, 596.
Bass, C. C.: Med. Rec., Sep. 24, 1910, 78, 538.
Galambos, A.: Wien. klin. Woch., 8 Dec. 1910, 23, 1748.
Kakowski, A.: Deutsch. med. Woch., Dec. 22, 1910, 36, 2391.
Müller, F.: E. Abderhalden's Handbuch der biochemischen Arbeitsmethoden, Berlin and Vienna, vol. 3, 1910, p. 713.
Fuhrmann, F.: Ibidem, p. 1278.
Klemensiewicz: Idem, p. 1278.
Kempff, R.: Id., 1, 25.

1911

Permin, C.: Hospitalstidende, 18 Jan. 1911, 54, 63.
Howard, B. J.: Circ. no. 28, Bureau of Chemistry, U.S. Dept. Agriculture, Feb. 13, 1911, p. 4.
Sutton, W. S.: Jour. Am. Med. Assn., Mch. 11, 1911, 56, 737.
Dunger, R.: Münch. med. Woch., 23 May 1911, 58, 1133.
Schmincke, R.: Münch. med. Woch., May 23, 1911, 58, 1134.
Schilling, V.: Deutsch. med. Woch., 22 June, 1911, 37, 1159.
Katzenstein, M. B.: Jour. Am. Med. Assn., July 1, 1911, 57, 22.
Schilling-Torgau, V.: Folia haematologica, 4 July 1911, 12, Archiv, 130.
Pitfield, R. L.: N. Y. Med. Jour., July 8, 1911, 94, 96.
Geissler, W.: Münch. med. Woch., 5 Sep. 1911, 58, 1917; also 31 Oct. 1911, 58, 2327.
Schilling, V.: Zeitschr. f. exp. Pathol. und Therapie, 24 Nov. 1911, 9, 687.
Hirschfeld, H.: Berl. klin. Woch., 4 Dec. 1911, 48, 2209.
Hirschfeld, H.: Folia haematologica, 1911, 12, 347.
Thomson, D.: Annals of Tropical Medicine, Dec. 30, 1911, 5, 3.
Rayleigh, .: Interference of Light, in Encycl. Brit., Cambridge, England, and New York, ed. 11, 1911, p. 685.

1912

Morawitz, P.: Zeitschr. f. ärztl. Fortbildung, 1 Jan. 1912, 9, 1.
Schilling, V.: ibidem, p. 26.
Hill, R. A. P.: Lancet, Jan. 6, 1912, 1, 20.
Schall, M.: Deutsch. med. Woch., 11 Jan. 1912, 38, 74.
Hastings, T. W.: Medical Times, Jan. 1912, 40, 2.
Thomson, D.: Lancet, Feb. 17, 1912, 1, 438.
Thomson, D.: Medical Press and Circular, Apr. 24, 1912, n.s. 93, 432.
Sheaff, P. A.: Jour. Am. Med. Assn., June 22, 1912, 58, 1941.
Ellermann, V.: Ugeskrift for Laeger, June 27, 1912, 74, 957.
Pappenheim, A.: Deutsche med. Wochenschrift, 31 Oct. 1912, 38, 2067.

Bürker, K.: in R. Tigerstedt's Handbuch der physiologischen Methodik, Leipzig, 2, Abt. 5, 1912, 157.
Morawitz, P : in L. Mohr' and R. Staehelin's Handbuch der inneren Medizin, Berlin, 4, 1912, 92.
Klyuchareff, S. I.: Prakt. Vrach, 1912, 11, 1.
Von Domarus,; Taschenbuch d. klin. Hämatologie, Leipzig, 1912, p. 5.
Wright, A. E.: Technique of the Teat, London, 1912.

1913

Ellermann, V.: Deutsch. Arch. f. klin. Med., 16 Jan. 1913, **109**, 378.
Zapf, L.: Med. Klinik, 2 Feb. 1913, **9**, 170.
Maddox, R. D.: Jour. Am. Med. Assn., March 1, 1913, **60**, 663.
Lenzmann, R.: Med. Klinik, 13 Apr. 1913, **9**, 587.
Bürker, K.: Verhandl. d. Kong. f. innere Med., 17 Apr. 1913, **30**, 285.
Watters, W. H.: Jour. Am. Med. Assn., May 17, 1913, **60**, 1516.
Roerdansz, W.: Arch. f. d. ges. Physiologie, 10 June 1913, **152**, 81.
Glaubermann, J. A.: Neurol. Zentralbl., 16 June 1913, **32**, 750.
Bürker, K.: Arch. f. d. ges. Physiologie, 30 June 1913, **152**, 271.
Bürker, K., Jooss, E., Moll, E., and Neumann, E.: Zeitschr. f. Biol., 26 July 1913, **61**, 379.
Bürker, K.: Arch. f. d. ges. Physiologie, 31 July 1913, **153**, 128.
Von Putkowski, .: Med. Klinik, 4 Aug. 1913, **9**, 1380.
Krotkow, S. F.: Arch. f. d. ges. Physiologie, 15 Sep. 1913, **153**, 616.
Schilling, V.: Deutsch. med. Woch., Oct. 9, 1913, **39**, 1985.
Jörgensen, G.: Ugeskrift for Laeger, Oct. 30, 1913, **75**, 1753.
Eustis, A.: Jour. Am. Med. Assn., Nov. 29, 1913, **61**, 1984.
Rea, C.: Jour. Am. Med. Assn., Dec. 27, 1913, **61**, 2312.
Barnett, C. H. J.: ibidem, 2312.
Kraemer, W. H.: idem, 2312.
Champy, C.: Le Sang, Paris, 1913.

Emerson, C. P.: Clinical Diagnosis, Phila. and London, ed. 4, 1913, 447, 460, 462.
Morris, R. S.: Clinical Laboratory Methods, New York, 1913, 239.
Weidenreich, F.: in A. Gilbert and M. Weinberg's Traité du sang, Paris, 1913, **1**, 4.

1914

Schroeder, F. B.: Jour. Am. Med. Assn., Feb. 7, 1914, **62**, 455.
Goriaeff, N. K.: Russky Vrach, Feb. 8, 1914, **13**, 181.
Roerdansz, W.: Folia haematologica, 24 Feb. 1914, **18**, 1.
Nieuwenhuijse, P.: Nederlandsch. Tijdschrift v. Geneesk., 21 Mch. 1914, **58**, 872.
Callison, J. C.: Jour. Am. Med. Assn., Apr. 4, 1914, **62**, 1086.
Metz, C.: Münch. med. Woch., 5 May 1914, **61**, 991.
Schirokauer, H.: Berl. klin. Woch., 18 May 1914, **51**, 936.
Johns, F. M.: New Orleans Med. and Surg. Jour., Oct. 1914, **67**, 332.
Roerdansz, W.: Deutsch. med. Woch., 12 Nov. 1914, **40**, 1962.
Jörgensen, G.: Zeitschr. f. klin. Med., 1914, **80**, 21.
Van Walsem, G. C.: Zeitschr. f. wissenschaftliche Mikroskopie, 1914, **31**, 310.

Croy, C. C.: in Webster, p. 433.
Gulland, G. L., and Goodall, A.: The Blood, Edinburgh and London, ed. 2, 1914, 4.
Sahli, H.: Lehrbuch der klinischen Untersuchungs-Methoden, Leipzig und Wien, ed. 6, 1914, **2**, 265, 308, and 318.
Webster, R. W.: Diagnostic Methods, Phila., ed. 3, 1914, 383; also ed. 6, 1920.
Wright, A. E.: Technik von Gummisaugkappe und Glaskapillare, Jena, 1914, pp. 3, 25, 31; translation of Technique of the Teat, London, 1912.

1915

Cummer, C. L., and Dexter, R.: Jour. Am. Med. Assn., Feb. 13, 1915, **64**, 584.
Hartz, H. J.: New York Med. Jour., Mch. 27, 1915, **101**, 612.
Metz, C.: Deutsch. med. Woch., 8 July 1915, **41**, 825.
Bass, C. C.: Jour. Am. Med. Assn., Sep. 18, 1915, **65**, 1028.
Van Walsem, G. C.: Deutsch. med. Woch., 30 Sep. 1915, **41**, 1193.
Wells, J. J., and Sutton, J. E.: Am. Jour. Physiol., Nov. 1, 1915, **39**, 31.
Williams, B. G. R., and Williams, E. G. C.: Laboratory Methods, St. Louis, ed. 3, 1915, p. 57.

1916

Liebreich, E.: Deutsch. med. Woch., 13 Apr. 1916, **42**, 453.
Kennedy, W. T.: Official Gazette of U. S. Patent Office, May 9, 1916, **226**, 485.
Epstein, A. A.: Jour. Lab. and Clin. Med., May 1916, **1**, 610.
Morris, R. S.: Jour. Lab. and Clin. Med., Dec. 1916, **2**, 208.

1917

Little, G. E.: Jour. Lab. and Clin. Med., Jan. 1917, **2**, 264.
Levy, M.: Official Gazette of U. S. Patent Office, Jan. 30, 1917, **234**, 1497.
Lyon, M. W.: Jour. Am. Med. Assn., Mch. 3, 1917, **68**, 709.
Stein, I. F.: Jour. Am. Med. Assn., Aug. 4, 1917, **69**, 383.
Diner, J.: Jour. Am. Med. Assn., Oct. 27, 1917, **69**, 1421.
Levy, A. G.: Brit. M. J., Dec. 1, 1917, **2**, 715.
Greene, C. L.: Medical Diagnosis, Phila., ed. 4, 1917, 112–133.
Wood, F. C.: Chem. and Microscopical Diagnosis, New York, ed. 3, 1917, 36 and 45.

1918

Gehrmann, A.: Illinois Med. J., Jan. 1918, **33**, 30.
Drinker, C. K.; Drinker, K. R., and Kreutzmann, H. A.: J. Exp. Med., Feb. 1, 1918, **27**, 249.
McJunkin, F. A., and Charlton, A.: Arch. Int. Med., Aug., 1918, **22**, 157.
Bürker, K.: Med. Klinik, 24 Nov. 1918, **14**, 1174.
Liot, A., and Poussin, M.: Bull. des sci. pharmacol., 1918, **25**, 23.

Frazier, C. H.: Surgery of the Spine, N. Y. and London, 1918, p. 177.
Hawk, P. B.: Practical Physiological Chemistry, Phila., ed. 6, 1918, 309.
Simon, C. E.: Clinical Diagnosis, Phila., ed. 9, 1918, 74.
Todd, J. C.: Clinical Diagnosis, Phila. and London, ed. 4, 1918, 252.

1919

Hirschfeld, H.: Zeitschr. f. aerztl. Fortbild., February 1, 1919, **16**, 65.
Putnam, T. J.: Jour. Am. Med. Assn., Feb. 22, 1919, **72**, 571.
Aebly, J.: Correspondenz-Blatt f. Schw. Aerzte, 12 Apr. 1919, **49**, 478.
Alder, A.: ibidem, 21 June 1919, **49**, 925.
Rohrer, F.: idem, 28 June 1919, **49**, 971.
Spindler, F. G., assignor to E. Leitz Inc.: Official Gazette U. S. Patent Office, Aug. 26, 1919, **265**, 483.
Marloff, R.: Arch. f. d. ges. physiologie, 1919, **175**, 355.
Zeller, H.: Zeitschr. f. d. ges. exp. Med., 1919, **10**, 103.

Martinet, A.: Diagnostic Clinique, Paris, 1919, p. 219.
Pappenheim, A.: Technik der klin. Blutuntersuchung, Leipzig, ed. 2, 1919.
McJunkin, F. A.: Clinical Microscopy, Phila., 1919, p. 23.
Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Berlin, ed. 3, 1919.

1920

Harvey, W. F.: Indian Jour. Med. Research, January, 1920, **7**, 479.
Reichart, A.: Wien. klin. Woch., January 8, 1920, **33**, 45.
Gram, H. C.: Arch. Int. Med., March, 1920, **25**, 325.
Gifford, S. R.: Jour. Am. Med. Assn., April 10, 1920, **74**, 1024.
Degkwitz, R.: Folia haematologica, June 1920, **25**, 153.
Meulengracht, E.: Ugeskr. f. Laeger, June 3, 1920, **82**, 715; abstr. in Jour. Am. Med. Assn., July 24, 1920, **75**, 282.
Gram, H. C.: Ugeskr. f. Laeger, June 3, 1920, **82**, 718; abstr. in Jour. Am. Med. Assn., July 24, 1920, **75**, 282.
Kilgore, E. S.: Jour. Am. Med. Assn., July 10, 1920, **75**, 86.
Haden, R. L.: Jour. Lab. and Clin. Med., July 1920, **5**, 672.
Shilling, V.: Berl. klin. Woch., September 20, 1920, **57**, 895.
Harvey, W. H.: Brit. Med. Jour., September 25, 1920, **2**, 480.
Bierring, K.: Ugeskr. f. Laeger, November 18, 1920, **82**, 1445.
Agasse-Lafont, E.: Gaz. d. hôp., 1920, **93**, 154.
Alder, A.: Zeitschr. f. klin. Med., 1920, **88**, 74.
Fritsch, G.: Arch. f. d. ges. Physiologie, 1920, **181**, 80.
Thomsen, O.: Acta Med. Scandinav., 1920, **53**, 507.
Gram, H. C.: Acta Med. Scandinav., 1920, **54**, 1.
Thomsen, O.: Zentralbl. f. Herz und Gefässkrankh., 1920, **12**, 145.

Von Rohr, M.: Die binokularen Instrumente, Berlin, ed. 2, 1920, pp. 80, 128, 163, 192, 224, 265.

1921

Dreyer, G.: Lancet, January 29, 1921, **1**, 219.
Gray, H.: Jour. Am. Med. Assn., June 25, 1921, **76**, 1826.